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A Food-Poisoning Outbreak of Multiple Pathogenic Factors

1. Introduction

A food-poisoning outbreak affecting many teachers and school children occurred on 12 March 1993 in the Yenpu Primary School of Pingtung County, southern Taiwan. The investigation included: questionnaire survey, assessment of the kitchen and the food processing, and testings of specimens of foods and from humans. Of the 699 valid questionnaires, 160 (23%) showed symptoms of abdominal pain, dizziness, flushing, and diarrhea. Incubation periods ranged from 10 minutes to more than 10 hours. Both *Bacillus cereus* and histamine were isolated from food specimens. Soybean milk, sweet and sour fish and fried vegetables were considered risk factors of the outbreak. The outbreak reminds us that: 1) a food-poisoning outbreak could be induced by many pathogenic factors at the same time; and 2) the incubation period and symptoms of *Bacillus cereus* poisoning could vary greatly.

While more people are eating outside, the number of food-poisoning victims has been increasing, so effective control of food-poisoning has become an important public health issue. Poor storage of foods, inadequate cooking temperature, contamination of facilities and processing are some major causes of food-poisoning. Food-poisonings induced by single pathogenic factor are more common while outbreaks caused by multiple factors are infrequent. The present outbreak was an unusual one induced by multiple pathogenic factors.

2. Background

On 13 March 1993, newspapers reported a food-poisoning outbreak on 12 March in the Yenpu Primary School of Pingtung County. Of a total of 764 teachers and students, some had the soybean milk prepared by the school at 10 am. Two hours later, 10 or so teachers and students developed symptoms of abdominal pain, dizziness and nausea. School lunch of sweet and sour fish, fried vegetables, rice and soup was served at noon. After lunch, some one hundred teachers and students developed symptoms of

dizziness, flushing, abdominal pain and palpitation. As the number of cases affected was relatively large and the symptoms varied, as well as the outbreak also involved the sanitation of school kitchen, an investigation was thus undertaken.

3. Materials and Methods

Since 13 March was a Saturday, the investigation, with the assistance of the local health bureau, began on 15 March.

Copies of a self-administered questionnaire was distributed to all teachers and students of the Yenpu Primary School. The questionnaire included: personal background information, food items and amounts consumed on the day, time of consumption, symptoms if any, time of the onset of symptoms, types of symptoms, medication or hospitalization, and date of recovery. Twenty some teachers and students with symptoms were interviewed individually.

A case was defined as: a teacher or a student of the school who had developed, between the mornings of 12 and 15 March, one of the symptoms such as abdominal pain,vomiting or flushing, or two or more such symptoms as nausea, diarrhea, dizziness, palpitation or itching of skin.

The kitchen was inspected. Cooks, teachers in charge and students were asked about the process in which foods were prepared on 12 March.

Leftovers of lunch: the sweet and sour fish, fried vegetables, rice and soybean milk were tested by the Southern Center of the National Laboratories of Foods and Drugs. Human specimens were also collected: vomituses of two students and rectal swabs of three cooks were sent for testings by the Southern Center of the National Institute of Preventive Medicine.

For food specimens, only *Staphylococcus aureus* and *Bacillus cereus* were tested; the amount of histamine in foods was also measured. Routine testings for *Vibrio cholerae, Vibrio parahaemolyticus, Salmonella, Shigella,* and *Staphylococcus aureus* were conducted with human specimens.

Epi-Info 5.0 and SAS were used for statistical analyses of relative risks. X^2-test, Mantel-Haenszel X^2-test and logarithmic multiple regression were applied for significance test.

4. Findings

Of the 699 valid questionnaires collected, 160 (23%) met the criteria of a case. Of them, 124 had been medicated and six hospitalized. As shown in the Fig. 1, incubation periods ranged from 10 minutes to more than 10 hours,though most had recovered within one or two days. Cases were placed in three groups by the time of onset for

further analyses: before noon in the first group, between noon and 4 pm. in the second group, and after 4 pm. in the third group.

Distributions of symptoms are shown in Tables 1 through 4. Major symptoms are abdominal pain, dizziness and flushing. By group, major symptoms of the first group are abdominal pain and dizziness; of the second group, dizziness, flushing and abdominal pain; and of the third group, abdominal pain, dizziness and diarrhea.

X^2-testing of the relation between onset of disease and food items shows significant relationships between onset and the soybean milk, and the sweet and sour fish and fried vegetables of the lunch (see Table 5). By group, for the first group, soybean milk is found highly related (RR=80.6, P=0.000), and the soup, negatively related (RR=0.25, P=0.015) (see Table 6); for the second group, the sweet and sour fish (RR=11.7, P=0.002) and the soup (RR=4.37, P=0.026) are found related (see Table 7), though further analyses by stratified analysis and logarithmic multiple regression show that only the sweet and sour fish is related; for the third group, the fried vegetables are found related (RR=3.67, P=0.010) (see Table 8).

Laboratory testings have identified 6.4×10^6 cfu/gm and 2.0×10^5 cfu/gm of *Bacillus cereus* in the soybean milk and fried vegetables respectively; and 24.5 mg% of histamine in the sweet and sour fish. Test findings of vomituses and rectal swabs are all negative. It was found later that the two students whose vomituses were sent for testings had consumed only the sweet and sour fish, rice and soup but not the fried vegetables and soybean milk that day.

The kitchen has been newly built and fairly clean. Some defects are: the container for soybean milk smells, some aprons are dirty, and food processing area is not separated from the dining area.

The soybean milk was prepared at 110°C at 8 am. of 11 March, placed in a large container, and served to some students. The leftover was placed in small containers at 3 pm. of the same day and stored in the upper and lower portions of the refrigerator. It was served again at 10 am of 12 March.

On that day, the upper part of the refrigerator was freezing, the temperature of the lower part was not low enough. A few days later, the refrigerator was repaired, and the temperatures of the upper and lower parts reached −10°C and 0°C respectively, with a difference of 10°C.

Lunch of 12 March included: sweet and sour fish (swordfish, pineapple, onion, ketchup and carrot), fried vegetables (celery, bear sprout and shredded meat), soup (meat-bone, cucumber and spiced cabbage) and rice. Materials were delivered at 8 am. and prepared under room temperature. The sailfish was sliced at 10 am., flavored and fried by 11 am., and added sauces. Vegetables were fried in a large cooking pot of one meter in diameter and 0.5 meter in depth. They were cooked by 11 am..

The vegetable dealer said that the vegetables were bought from the market at 4 to 5

am. and that the fish was a cut of a large sailfish. The fish was still frozen when bought and was kept in room temperature throughout the processing.

Teachers with flushing said that the fish did not smell and that other food items tasted normal. Students of the class, many of whom had developed symptoms before lunch, said the soybean milk tasted sugar cane bagasse.

The temperature on 11 and 12 March in Pingtung was between 25°C and 30°C.

5. Discussion

The investigation raised several questions: why did the incubation periods show such a large gap of ten minutes to more than 10 hours? Why were the symptoms so varied? Why did some cases develop symptoms before lunch and why were their symptoms so different from symptoms of those who became sick after lunch? To answer these questions, the cases are divided into three groups by the time of onset.

Statistical analysis shows that onset of disease in the first group is related to the soybean milk, though the RR of the soup is 0.25. This is probably due to the fact that at the time of lunch, the patients were already feeling uncomfortable, they could no longer consume a large amount of soup. That symptoms appeared only a few minutes after the consumption of soybean milk suggests toxin as the likely agent. From utensils, the pH of foods and taste, chemical toxins could be eliminated. The poisoning was more likely due to becterial toxins such as toxins of *Staphylococcus aureus* and *Bacillus cereus.*

The soybean milk was safe a day before , and it was not heated again. Though soybean milk was not tested for *Staphylococcus aureus* toxin, culture findings for *Staphylococcus aureus* were negative. The likelihood of poisoning by *Staphylococcus aureus* toxin could thus be eliminated.

Bacillus cereus is a spore-forming bacillus. The optimal temperature for breeding and spore-forming is 30°C (between 7°C and 55°C). The heat resistance of the spores varies with serotypes[1]. It is likely that only certain serotypes could cause poisoning[2]. Traditionally, the poisonings are grouped into vomiting and diarrheal types. The vomiting type of which medium of transmission is rice has an incubation period of one to six hours with vomiting as its major symptom, and diarrhea only occasionally. The diarrheal type has an incubation period of eights to 16 hours with abdominal pain, diarrhea and nausea as its major symptoms. Review of literature[3-5] shows that both the incubation period and the symptoms of the incident are not typical. For the vomiting type, Mortimer and McCann[3] report that patients develop diarrhea one to four hours after vomiting; and some patients develop nausea and abdominal pain instead of vomiting[4]. Vomiting type has also been found in media other than rice[4]. As for the diarrheal type, in experiments by inducing poisoning in monkeys, the incubation period is found to be only 0.5 to 3.5 hours[5]. In some cases, nausea precedes vomiting[6].

Laboratory testings have isolated 6.4×10^6 cfu/gm of *Bacillus cereus* in the soybean milk specimens but not in the human specimens. From the epidemiological point of view, the incident is considered more likely a food-poisoning induced by soybean milk contaminated by *Bacillus cereus*. It is of vomiting type in that patients developed symptoms within minutes; though on the other hand, it is more of the diarrheal type in that the symptoms were primarily abdominal pain and nausea and less vomiting, and diarrhea occurred four to eight hours later. Whether this incident is of vomiting type with less vomiting or of diarrheal type with shorter incubation period required further investigation. However, when a food-poisoning outbreak of shorter incubation period is encountered, whether the medium involved is rice or others and whether the major symptom is vomiting or else, the possibility of *Bacillus cereus* poisoning should always be considered.

The mode of contamination could be: 1. the soybean milk was not heated enough to kill the spores of *Bacillus cereus* in the soybeans; 2. the soybean milk was left in room temperature too long (around six to seven hours); 3. the temperature of the lower part of the refrigerator was not low enough, and *Bacillus cereus* in the containers started to breed

Some developed allergy-like flushing soon after lunch. However, the number of cases was large; and a cook who had lunch at school did not develop any symptoms, but soon after he and his family members shared the fried sailfish he brought home, they all developed symptoms such as flushing, palpitation and numbness of mouth. These indicate the possibility of scombrotoxic poisoning. Statistical analysis of the questionnaires also shows that the onset of disease of the second group is related significantly to the sweet and sour fish.

Scombrotoxic poisoning is caused by the histamine and saurine in the fish meat, primarily of mackerels, and produced through the process of decarboxylation due to inadequaate storage under a temperature of 20°C to 30°C. The acceptable level of histamine in fish is less than 5 mg%, and when the level is higher than 20mg%, poisoning could occur. When the concentration of histamine is higher than 100 mg%, poisoning always occurs[7]. Fish meat that is likely to induce poisoning tastes normal. Other fishes of dark-colored meat can also induce poisoning. An incident of scombrotoxic poisoning induced by swordfish in Taiwan was reported in November 1992. The histamine concentration in swordfish could reach as high as 831 mg%, higher than the 486 mg% of the migratory fish (*Coryphaena* spp.) of Hawaii that often induces scombrotoxic poisoning[8].

Reason for this scombrotoxic poisoning could be that the swordfish was not properly stored after being caught, though it could never be proved. The fact that the fish was kept in room temperature for six hours could cause decay of the outer layer of fish meat. This could explain the fact that only some of those who had had the fish became ill.

Scombrotoxic poisoning often starts in 10 minutes to 2−3 hours[9]. However, between the period of 16 hours of the day and noon of next day, 40 some individuals

still developed symptoms such as abdominal pain, dizziness and diarrhea, which were quite different from the flushing of the second group and many of them did not have the soybean milk. A third pathogenic factor was thus suspected. Two of those who became sick 5−6 hours after lunch had only fried vegetables, soup and rice, but not the sweet and sour fish and soybean milk. It is likely that cases in the third group became ill within a few hours, though their symptoms were mild and very few had high fever. The possibility of diarrheal type *Bacillus cereus* or *Clostridium perfringens* poisoning was suspected. From the statistical analysis of questionnaires, the onset of disease was found to be related to fried vegetables. *Clostridium perfringens* are often found in vegetables[9]. Cases of *Bacillus cereus* food poisoning resulting from eating raw vegetable sprouts have been reported[6].

Though some 2.0×10^5 cfu/gm of *Bacillus cereus* have been isolated in the fried vegetables, they are quite common in foods[10]. Regretfully, there were no human specimens to verify. *Clostridium perfringens* were not tested for. Under those circumstances, though it was not possible to prove that *Bacillus cereus* was the agent, the possibility of *Clostridium perfringens* poisoning could not be eliminated. Had the laboratories tested the food specimens for total colonies, it would have been very helpful to the readings of the findings, as the item of bacillus for testing was few.

Vegetables were contaminated probably because the amount was so large to be cleaned properly, particularly the bean sprouts and celeries. The heat transmission of the cooking pot was not even, and a part of it was not heated enough to kill the agents. The same conditions would have been there before, and yet no food poisoning outbreaks had been reported. Perhaps the number of cases was so small to be noticed. Further investigations are thus required.

6. Conclusions

We suspect that the present incident was induced by three pathogenic agents: *Bacillus cereus* (either the vomiting or the diarrheal types), scombrotoxic poison and another unknown agent.The major reason for the outbreak was the inadequate storage of foods.

7. Recommendations

We have learned from the present outbreak that food poisoning could be induced by many pathogenic agents at the same time. During investigations, one should never jump to a conclusion of one agent. More thorough investigations will provide us more information for the planning of more comprehensive preventive measures.

We should also recommend that cooking and food handling staff of schools should be trained on food sanitation and personal hygiene.

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References:

1. Bradshaw JG, Peeler JG, Twedt RM. Heat resistance of ileal loop reactive *Bacillus cereus* strains isolated from commercially canned food. Appl. Microbiol 1975; 30:943–945.
2. Taylor AJ, Gilbert RJ. *Bacillus cereus* food poisoning: a provisional serotyping scheme. J Med Microbiol 1975; 8:543–550.
3. Mortimer PR, McCann G. Food-poisoning episodes associated with *Bacillus cereus* in fried rice. The Lancet May 25, 1974; 1043–1045.
4. Holmes JR, Plunkett T, Pate P. Endemic food poisoning caused by *Bacillus cereus*. Arch Intern Med 1981; 141:766–767.
5. Gilbert RJ, Kramer JM. *Bacillus cereus* entertoxins; present status. Biochemical Society Transactions 604th Meeting, Cambridge.
6. Portnoy BL, Goepfert JM, Harmon SM. An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. Am J Epidemiol 1976; 103:589–594.
7. Bartholomew BA, Berry PR, Rodhouse JC et al. Scombrotoxic food poisoning in Britain; feature of over 250 suspected incidents from 1976 to 1986. Epi Inf 1987; 99: 775–782.
8. Suyama M, Yoshizawa Y. Free amino acid composition of the skeletal muscle of migratory fish. Bull Jap Soc Sci Fish 1973; 99: 775–782.
9. Barnam AH, Evans MG. Foodborne pathogens an illustration text 1991. Wolfe Publishing Ltd, England.
10. Turnbull PCB, Kramer JM. Intestinal carriage of *Bacillus cereus*; faecal isolation studies in three populations. J Hyg Camb 1985; 95: 629–638.

Table 1. Distribution of symptoms — All cases (160 persons)

Symptoms	No.	%
Abdominal pain	112	70.0
Dizziness	106	66.3
Flushing	69	43.1
Diarrhea	46	28.8
Nausea	43	26.9
Self-felt fever	39	24.4
Palpitation	38	23.8
Weakness	33	20.6
Vomiting	20	12.5
Itching of skin	11	6.9
Others	8	5.0

Table 2. Distribution of symptoms — Cases becoming ill before noon (18 persons)

Symptoms	No.	%
Abdominal pain	16	88.9
Dizziness	13	72.2
Nausea	5	27.8
Weakness	5	27.8
Flushing	5	27.8
Diarrhea	4	22.2
Vomiting	4	22.2
Self-felt fever	3	16.7
Palpitation	0	0
Itching of skin	0	0
Others	0	0

Table 3. Distribution of symptoms — Cases becoming ill between noon and 4 pm. (94 persons)

Symptoms	No.	%
Dizziness	67	71.3
Flushing	61	64.9
Abdominal pain	54	57.4
Palpilation	33	35.1
Self-felt fever	32	34.0
Nausea	29	30.9
Diarrhea	22	23.4
Weakness	22	23.4
Vomiting	11	11.7
Itching of skin	9	9.6
Others	7	7.4

Table 4. Distribution of symptoms — Cases becoming ill after 4 pm. (44 persons)

Symptoms	No.	%
Abdominal pain	38	86.4
Dizziness	25	56.8
Diarrhea	18	41.0
Nausea	9	20.5
Weakness	6	13.6
Vomiting	5	11.4
Palpitation	5	11.4
Self-felt fever	3	6.8
Flushing	3	6.8
Itching of skin	2	4.5
Others	1	2.3

Table 5. Food items and onset of disease

Food items	Became ill		Not ill		RR#	P-value
	Eaten	Not eaten	Eaten	Not eaten		
Soybean milk	45	115	78	461	1.83	0.000*
Sweet-sour fish	153	7	465	71	2.76	0.003*
Fried vegetable	134	25	374	160	1.95	0.001*
Soup	151	9	484	52	1.61	0.150
Rice	154	6	507	29	1.36	0.524

RR = relative rish * p < 0 05

Table 6. Food items and onset of disease before 12 noon

Food items	Became ill		Not ill		RR#	P-value
	Eaten	Not eaten	Eaten	Not eaten		
Soybean milk	17	1	104	573	80.60	0.000*
Sweet-sour fish	14	4	600	74	0.44	0.134
Fried vegetable	13	4	491	181	1.19	0.000*
Soup	13	5	619	55	0.25	0.015*
Rice	15	3	642	32	0.27	0.057

RR = relative rish * p < 0 05

Table 7. Food items and onset of disease between noon and 4 pm.

Food items	Became ill		Not ill		RR#	P-value
	Eaten	Not eaten	Eaten	Not eaten		
Soybean milk	16	78	105	496	0.97	0.969
Sweet-sour fish	92	1	522	77	11.70	0.002*
Fried vegetable	77	17	427	168	1.66	0.053
Soup	92	2	540	58	4.37	0.026*
Rice	91	3	566	32	1.61	0.610

RR = relative rish * p < 0 05

Table 8. Food items and onset of disease after 4 pm.

Food items	Became ill		Not ill		RR#	P-value
	Eaten	Not eaten	Eaten	Not eaten		
Soybean milk	10	34	111	540	1.40	0.450
Sweet-sour fish	42	2	572	76	2.67	0.215
Fried vegetable	40	4	464	181	3.67	0.010*
Soup	43	1	589	59	4.08	0.165
Rice	44	0	613	35	—	0.158

RR = relative rish * p < 0.05

Fig. 1. Time of onset of food poisoning, Yenpu Primary School, Pingtung County